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(54) Title: PRODRUGS OF THALIDOMIDE AND METHODS FOR USING SAME AS MODULATORS OF T-CELL FUNCTION

(57) Abstract

The present invention relates to a new, safe and effective form of thalidomide ([N-phthalimido]-glutarimide) and methods of using the same. More specifically, the invention relates to prodrugs of thalidomide and prodrugs of certain analogs of thalidomide, which comprise a thalidomide or analog component having bound thereto the dipeptide sequence X-pro, wherein "X" is one of a wide variety of amino acids and "pro" represents the imino acid proline.

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PRODRUGS OF THALIDOMIDE AND METHODS FOR USING SAME AS MODULATORS OF T-CELL FUNCTION

REFERENCE TO RELATED APPLICATIONS

The present invention claims priority to U.S. Provisional Application No. 60/018,558, filed May 29, 1996, entitled PRODRUGS OF THALIDOMIDE AS MODULATORS OF T-CELL FUNCTION, which is incorporated by reference herein in its entirety.

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to a new, safe and effective form of thalidomide ([N-phthalimido]-glutarimide) and methods of using the same. More specifically, the invention relates to prodrugs of thalidomide and prodrugs of certain analogs of thalidomide, which comprise a thalidomide or analog component having bound thereto the dipeptide sequence X-pro, wherein "X" is one of a wide variety of amino acids and "pro" represents the imino acid proline.

Discussion of Related Art

A great deal of excitement resulted from discoveries relating to the beneficial effects of thalidomide in treating a variety of diseases including leprosy, HIV-infection and rheumatoid arthritis. Thalidomide was first described in 1953 by Ciba and subsequently marketed by Chemic Grunenthal as an anticonvulsant for the treatment of epilepsy. The drug proved ineffective for this purpose; nevertheless it did induce good sleep. It achieved wide use in Europe as a "safe" alternative to barbiturates and later was used as an anti-emetic agent in pregnant women. The

compound appeared so nontoxic in rodent models that an LD50 could not be established. In 1961, however, limb defects in babies from mothers using thalidomide during pregnancy were described. Thalidomide proved to be a potent teratogen and was removed from the market due to extreme concerns about the threat of handicapping new generations of people by its teratogenic side effects.

Shortly thereafter, however, Sheskin et al. used thalidomide as a sedative in male leprosy patients experiencing erythema nodosum leprosum (ENL). Sheskin et al. (1965), Thalidomide in the treatment of lepra reactions. Clin Pharmacol. Ther. 6:303. To his surprise the clinical signs and symptoms of ENL were attenuated within 48 hours. Thalidomide's efficiency in ENL has been established beyond doubt in double-blind clinical trials.

More recently, thalidomide has also been used for rheumatoid, sarcoidosis, systemic lupus erythematosus, Behcet's disease, in acute and chronic graft vs. host disease (GVHD) following bone marrow transplantation and as an immunosuppressant agent in preventing cardiac allograft rejection. The drug has also lately become the object of wide-ranging research for its proposed value in treating a number of AIDS-related conditions, including aphthous ulcers, wasting, tuberculosis, as well as for treating HIV infection itself and the loss of functional CD4 T cells.

The rationale behind the use of this drug in HIV disease is somewhat involved. Rather paradoxically, thalidomide appears to work by pacifying or down regulating a part of the immune response, a property which would not seem to benefit a disease described in an immune deficiency. Excessive levels of TNFa found in HIV infection promote the development of aphthous ulcers, dementia, fevers, fatigue and wasting. How thalidomide up-regulates IL-2 and down-regulates INTa, is not known, although an interaction of thalidomide with the cytokine receptor assembly has been proposed. Recently, Shannon et al. reported that

thalidomide can stimulate the synthesis of IL-2 *in vitro*. Shannon et al., (1995), Thalidomide enhanced the production of IL-2 in cultures of human mononuclear cells stimulated with concanavalin-A, Streptococcal enterotoxin A and purified protein derivatives. Immunopharm. In this study mononuclear cells from seven healthy donors exposed to thalidomide and then stimulated with concanavalin-A (Con-A) secreted 187%±32% more IL-2 than control cultures stimulated with Con-A. Thalidomide also significantly increased the quantity of intracellular IL-2 in cells stimulated with Con-A or SEA (Staphylococcal enterotoxin-A) (Shannon and Smith (Studies conducted in October 1995, Baton Rouge, Louisiana)). If thalidomide is capable of facilitating IL-2 synthesis *in vivo*, especially in individuals prone to be deficient in IL-2 (i.e. HIV induced immunosuppression), this could be the drug's most beneficial effect. However, the extreme side effects linked to thalidomide, including the above-described teratogenic side effects and the potentially irreversible side effect of peripheral neuropathy, continue to hinder the widespread development of thalidomide treatments utilizing its proven beneficial attributes.

In view of the background set forth above, the present invention provides prodrugs of thalidomide (and prodrugs of certain analogs of thalidomide), which preferably comprise a thalidomide component having bound thereto a dipeptide leaving group. A dipeptide selected according to the invention preferably has the sequence X-pro, wherein "X" is one of a wide variety of amino acids and "pro" represents the imino acid proline. Inventive thalidomide prodrugs are inactive until metabolically altered by, for example, a site-specific enzyme, which activates the thalidomide component by removing the dipeptide therefrom. Also provided are methods of making inventive prodrugs and methods of using the same to treat a patient suffering from, for example, aphthous ulcers, wasting, tuberculosis, dementia, fevers, fatigue, HIV infection and the loss of functional CD4 T cells.

SUMMARY OF THE INVENTION

To overcome problems in the prior art relating to extreme side effects caused by systemic administration of thalidomide to a patient, the present invention provides prodrugs of thalidomide, and methods for making and using the same. Prodrugs of the invention may be administered to a patient, thereby delivering an inactive form of thalidomide which subsequently may be activated in a site-specific manner. Thereby, beneficial drug activity is achieved at a target site without systemic delivery of the active drug.

In one aspect of the invention, there is provided a method for treating a patient comprising providing a composition which comprises a thalidomide prodrug and administering the thalidomide prodrug to the patient. The prodrug preferably comprises a thalidomide component having bound thereto a dipeptide comprising the sequence X-pro; wherein "X" is one of a wide variety of amino acids and "pro" represents the imino acid proline. In one preferred aspect of the invention, the composition comprises a thalidomide prodrug and a pharmaceutically acceptable carrier.

In another aspect of the invention, there is provided a composition useful for selectively treating Aphthous ulcers, wasting, tuberculosis, dementia, fevers, fatigue, HIV infection and the loss of functional CD4 T cells, comprising a thalidomide component having a dipeptide selected in accordance with the invention bound thereto. Also provided are methods for making these thalidomide prodrugs.

It is an object of the present invention to provide a method for delivering the proven beneficial drug thalidomide to a patent in the form of a prodrug made or selected in accordance with the invention, thereby reducing systemic residence of the active drug and, correspondingly, the risk

of extremely unsatisfactory side effects.

Additionally, it is an object of the present invention to provide a prodrug of thalidomide including therein a dipeptide leaving group having a proline imino acid in the penultimate location.

It is another object of the invention to provide a process for making inventive thalidomide prodrugs by binding two amino acids selected in accordance with the invention to a thalidomide molecule.

Further objects, advantages and features of the present invention will be apparent from the detailed description herein and the figures associated therewith.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

For the purposes of promoting an understanding of the principles of the invention, reference will now be made to specific embodiments and specific language will be used to describe the same. It will nevertheless be understood that no limitation of the scope of the invention is thereby intended, such alterations and further modifications in the invention, and such further applications of the principles of the invention being contemplated as would normally occur to one skilled in the art to which the invention pertains.

The present invention provides safe and efficacious compositions comprising derivatives of thalidomide or derivatives of a molecule substantially similar to thalidomide and having functionality substantially similar to thalidomide. The specific types of derivatives contemplated by the present invention are derivatives termed "prodrugs," this term being used herein to refer to an inactive form of a drug that can be enzymatically or physiologically converted to the active form. In preferred aspects of the invention, an inventive thalidomide derivative is a prodrug comprising a thalidomide component having bound thereto a "leaving group" which comprises a dipeptide or oligopeptide that is hydrolized from the thalidomide component by the specific activity of an enzyme.

The general concept of presenting a drug in a proform (prodrug) is not new. In reality "endogenous drugs" are stored as prodrugs until they are needed. The discovery by Dr. Donald Stiner in the late 1960's that insulin was synthesized and stored within the islet b cells as proinsulin and processed to an active hormone (active drug) by a specific protease was the first example of this phenomenon. However, the present invention is a novel use of the concept of prodrugs which finds extremely advantageous use in providing site-specific thalidomide

activity in a patient.

The invention also provides methods for using such thalidomide prodrugs to treat a number of AIDS-related conditions, including aphthous ulcers, wasting, tuberculosis, as well as for treating HIV infection itself and the loss of functional CD4 T cells. For purposes of describing the invention, the term "thalidomide" is intended to refer to thalidomide as well as substantially similar molecules having substantially similar functionality. In this regard, it is well known that many drugs and other molecules have non-active sites which may be chemically altered without substantially altering the functionality of active sites thereof. It is intended that the present invention encompass prodrugs of such thalidomide analogs which insubstantially differ from thalidomide with respect to structure and functionality.

In a preferred aspect, the invention relates to the highly site-specific activation of a prodrug to its active form, this activation being restricted to specific cell types where the drug is desired to provide pharmacological activity. In a preferred aspect of the invention, prodrug derivatives of thalidomide are provided which comprise a thalidomide component bound to a dipeptide leaving group which may be removed by a hydrolysis reaction catalyzed by an enzyme specific to a particular cell type. In one preferred aspect of the invention, the enzyme which activates the thalidomide prodrug is DPP-IV. DPP-IV is very substrate specific in its action (i.e. the motif of the peptide bond hydrolyzed), and is the only enzyme presently known to hydrolyze the peptide bond proximal to proline in the penultimate position to the N-terminal amino acid, releasing a free dipeptide. The biological function of DPP-IV has been well established as a processing protease; its action on proteins is to clip off the 90 degree right or left angle dipeptide to either activate or start the

deactivation of the substrate. According to the invention this biochemical mechanism is utilized to activate prodrug forms of thalidomide *in situ*.

Proline, the only mammalian imino acid, confers unique restraints on the conformation of a peptide chain, and many biologically important peptide sequences contain conserved site location prolines. Unlike the other amino acids, the a-nitrogen atom of proline is part of the rigid pyrolidine ring and, at the same time, is covalently bound by means of a secondary amine bond to the preceding amino acid. When present inside an a-helix, proline also sterically prevents the amide nitrogen of its C-terminal neighbor from making a hydrogen bond with a carboxyl in the preceding turn of the helix and thus introduces a kink of 20 degrees or more in the α-helix. Furthermore, unlike other amino acids, proline and hydroxyproline can more readily introduce structural heterogeneity since the amino acid preceding proline can adapt either the stereo isomeric cis or trans conformation with respect to proline.

The conformational restrictions imposed by proline motifs in a peptide chain appear to imply important structural or biological functions as can be deduced from their often remarkable high degree of conservation in many proteins and peptides, especially cytokines, growth factors and G-protein-coupled receptors. A remarkable number of cytokines share an X-pro sequence at their aminoterminus (X is one of a wide variety of amino acids). The N-terminal X-pro sequence may not only contribute to the biological activity, as has been demonstrated for IL-1 and IL-2, but also serve as a structural protection against nonspecific proteolytic degradation analogous to C-terminal amidation, acetylation, or N-terminal cyclization to pyroglutamic acid. It is believed that only the serine protease DPP-IV can cleave the Pro-Z peptide bond (the bond between proline and any amino acid except proline on the carboxy terminus

side of proline). The rate of hydrolysis is further affected by whether proline is in the cis or trans state and which amino acid is positioned to the right (Z); a pro-pro bond cannot be cleaved. It has also been suggested that prolyl isomerization may be involved in regulatory switching. In light of the above, it has been discovered that a X-pro dipeptide is an excellent leaving group for purposes of the present invention because it is selectively removed from the prodrug, thereby activating thalidomide, at specific anatomical locations where the enzyme DPP-IV is present. The above characteristics which proline impart to peptides is significant due to the recent advances made in the understanding of T cell subtypes and the functional integrity of their cytokine system.

DPP-IV is very site specific, thus providing an excellent pharmacokinetic profile with respect to thalidomide prodrugs of the present invention. The protease DPP-IV is an intrinsic transmembrane ectopeptidase of the serine class with its catalytic moiety on the extracellular side. It is a sialoglycoprotein; a homodimer anchored by a hydrophobic domain into the plasma membrane of T lymphocytes with a short intracytoplasmic tail of six amino acids. It is preferentially expressed on the cells with CD4* helper/memory (CDx29*) determinants. DPP-IV is a processing protease with a high substrate specificity toward dipeptides X-Pro at the N-terminal. The enzyme is able to bind and to hydrolyze extracellular cytokine substrates (E.G. Il-2, IL-6, IL-8, IFN, TNFa). DPP-IV is induced in the CL phase following mitogen activation of the T-cell and is essential for the transition from Cl to S phase of the cell cycle. In the course of T-cell stimulation in vitro, DPP-IV activity increases by a factor of 4 to 8 with peak activity at day 3 or 4. The expression of cell surface-associated DPP-IV parallels the capacity of the T-cell to produce IL-2. Active-site-specific agents to

inhibit the enzyme have shown that membrane bound DPP-IV plays a key role in T-cell mediated immune response.

As described above with respect to one aspect of the invention, a prodrug made and used according to the invention is prepared so that it is in an inactive state until a leaving group is cleaved from the prodrug, thereby rendering it active. In a preferred embodiment of the invention, the tissue specific enzyme DPP-IV elicits a localized response such as, for example, a cytokine response that can be therapeutic in HIV infection. While it is not intended that the present invention be limited by statements regarding mechanisms whereby it achieves its advantageous result, such a response is elicited by the activation of an inventive thalidomide prodrug.

According to a preferred aspect of the invention, a thalidomide prodrug is synthesized such that it comprises a thalidomide component bound to the dipeptide sequence X-pro, where "X" is one of a wide variety of amino acids and "pro" represents the imino acid proline. Synthesis of thalidomide prodrugs by preparing and binding two amino acids thereto may be achieved using procedures known in the art. Preferably, the prodrug includes a dipeptide selected from the group consisting of ala-pro (alanine, proline); lys-pro (lysine, proline) and gly-pro (glycine, proline). As described above, these dipeptide derivatives (prodrugs) are uniquely activated by the T-cell ectoenzyme, DPP-IV (dipeptidyl peptidase-IV, a component of CD26).

An inventive prodrug is advantageously used by administering the prodrug to a patient. In one aspect of the invention, a composition is provided which comprises an inventive thalidomide prodrug and a pharmaceutically-acceptable carrier, and the composition is administered to a patient, for example, orally or by injection. An inventive prodrug is a safe, novel and effective composition that can be used as a therapeutic

product to treat human immune modulated inflammatory conditions and infectious parasitic diseases; in particular HIV. According to a preferred aspect of the invention, upon administration to a patient, the X-pro dipeptide leaving group of the thalidomide prodrug is conveniently removed via hydrolysis by the ectoenzyme DPP-IV on the plasma membrane of activated monocytes and T-cells, thus releasing the active form of thalidomide proximal to the site of action. Thus, the thalidomide is substantially unavailable systemically, is less likely to be rapidly degraded, less sedative, less likely to have teratogenic side effects and could be more selectively targeted to the modulation of various cell types and cytokines, principally TNFa and TNFb, IL-1, IL-2, and IL-6.

While the invention has been described in detail in the foregoing description, the same is to be considered as illustrative and not restrictive in character, it being understood that only the preferred embodiments have been shown and described and that all changes and modifications that come within the spirit of the invention are desired to be protected. The invention will be further described with reference to the following specific Examples. It will be understood that these Examples are also illustrative and not restrictive in nature.

EXAMPLE ONE

Experimental Design and Methods

Synthesis

A commercially available amino-X synthon is treated with a suitable blocking agent to introduce a blocking group that will force subsequent cyclization to a five-membered ring. The blocked amino-X synthon is treated with a substitute phthalic anhydride. The ring is closed using any applicable dehydrating agent under conditions that will not destroy the blocking group. Any required transformations of the

substituents(s) on the aromatic ring are carried out be standard procedures taking care not to prematurely remove the blocking group. The blocking group is removed by one of the standard deblocking procedures chosen so as to avoid any deleterious effects to the phthaloyl group or any of its substituents. The peptide is attached either step-wise or as a unit using conventional peptide synthesis methods. Any blocking group on the peptide needed for its introduction is removed.

Biotinylated derivatives for determining cell surface binding are produced by:

a) suitably modifying the aromatic substituent on the phthaloyl moiety to accept a spacer,

b) attaching, by standard peptide synthesis methods, a four to twelve atom spacer containing blocked terminal amino group,

c) deblocking the spacer without deblocking the X moiety,

d) biotinylating by standard methods, and

e) deblocking at X and carrying out whatever further reactions are needed.

Culturing and Preparation of Cells

K562 and HL 60 leukemia cell lines are cultured in RPMI 1640 medium containing 10% fetal bovine serum, 0.5% HEPES, 2% glutamine, and 1% streptomycin/penicillin GIBCO (Grand Island NY). The cells are grown to a density of 2×10^5 cells/ml and subsequently harvested. For the culturing of peripheral blood lymphocytes (PBL), 20 ml of blood is drawn from healthy controls into vacutainers containing 5 ml of 1% NaFl (Becton/Dickinson). The blood is diluted with Hanks balanced salt solution (GIBCO) and layered over 50% Ficoll (GIBCO). The tubes are sedimented at room temperature for 30 minutes at 400 X g. The leukocyte layer is aspirated under sterile conditions after the supernatant has been

removed. The leukocytes are washed with 10 ml of Hanks balanced salt solution and placed in RPMI medium with 10% fetal calf serum, HEPES, glutamine, and P/S (GIBCO) at a concentration of 10^6 cells/ml. Phytohemagluttinin (GIBCO) is then added to a final concentration of 1%. The cells are maintained in culture in a humidified incubator with 5% CO_2 for 48 hours at 37°C and then harvested. The cells are then incubated with various prodrug forms of thalidomide.

Testing the efficacy of thalidomide derivative prodrugs

The ectoenzyme DPP-IV at the surface of T-cells is known to hydrolyze from the N-terminus of peptides residues containing ala-pro. A number of synthetic fluorogenic substrates have been produced which are effectively hydrolyzed by DPP-IV and can be used to verify activity of the enzyme on T lymphocytes and various cell lines used for the *in vitro* assays. The effectiveness of the T-cell DPP-IV to remove the residue ala-pro from the principal drug moiety, thalidomide is tested as follows. Briefly, a buffered suspension of the dipeptidyl derivatives of thalidomide is incubated with intact viable cells in HEPES buffered (pH 7.0) isotonic medium at 37°C for periods up to 30 minutes. The cells are then sedimented at 500 X g for 5 min at room temperature. The supernatant is extracted with methylene dichloride to extract free thalidomide. The methylene dichloride extracts are evaporated and taken up in 50 ml of methanol for HPLC analysis.

HPLC analysis of thalidomide derivatives

Buffers containing ala-pro-thalidomide are incubated in the presence of T-cells (or other cell lines) with measurable DPP-IV activity. The hydrolysis of the dipeptide derivatives are monitored by analytical HPLC. HPLC separation is done according to the method of Gross and Grutrer (1992), on a TSK gel ODS80TM column (TosoHaas,

Montgomeryville (sp PA) 250 x 4.6 mm i.d.) with a mobile phase of triethylamine phosphate, 0.01 M, pH 3.6 (solvent A) and acetonitrile (solvent B). Dicarboxylic compounds are rapidly degraded at neutral pH, but not at pH 3.6. Separation is accomplished by three step linear gradients of solvent B; 5% - 15 from 1 to 10 minutes, 15% - 25% from 10 - 20 minutes, and 25% - 55% from 20 to 30 min, all at a flow rate of 1 ml per min. Samples are analyzed on a Millinium 2010 HPLC system (Millipore Corp, Milford, MA) with a 996 photodiode array detector and a Hewlett-Packard 1046A programmable Fluorescence Detector. The identity of compounds is made by comparing the UV absorbance spectra with a library of thalidomide derivatives.

Assay of DPP-IV activity on T cells and other cell lines

Because thalidomide has been shown to modulate the level of CD26 on T cells (23), DPP-IV activity is monitored with synthetic fluorogenic substrates (Enzyme Systems Products, Dublin, CA). These substrates contain the Dipeptide ala-pro attached to a leaving group, trifluoromethylcoumarin. Trifluoromethylcoumarin is analyzed by a Fischer 4000 spectrofluorimeter. 2×10^6 cells are incubated with a mM ala-pro-trifluoromethylcoumarin in isotonic HEPES, pH 7.8 buffer for 2 to 30 min. The cells are sedimented at 500 x g for 5 min and the supernatant aspirated for fluorescence analysis. The activity of DPP-IV on T-cells is quantitated and correlated with the findings on the release of free thalidomide derivatives from the dipeptidyl prodrug by DPP-IV containing lymphocytes. Using monoclonal antibodies to quantitate DPP-IV levels on T lymphocytes, correlation is determined between levels of this enzyme with the level of binding and uptake of thalidomide derivatives.

Binding specificity of the prodrug or the released drug

Thalidomide derivatives are conjugated with biotin

through the R'-amino group of the phthalimide moiety. Biotin conjugates of the thalidomide derivative, once bound to or taken up by the target cell are coupled with added FITC-conjugated avidin and subsequently quantitated by flow cytometry. Quantitation of avidin-FITC is used to determine the binding of the thalidomide derivatives to target cells and analyze CD4 and/or other surface marker fluorescence (i.e. CD26) using PE-coupled monoclonal antibodies. Thus, binding/uptake of thalidomide derivatives in specific T-cell subsets is quantified. Specifically, 2×10^6 PHA-stimulated PBLs in 100 ml of PBL, pH 7.2, are incubated with 10 ml of avidin-FITC, 10 ml of CD-4-PE (phcoerythrin) and/or CD8-tricolor (Bectin Dickinson) (Dolbeare et al. 1996). The samples are washed with 5 ml PBL and sedimented at 500 x g for 5 min. The samples are then taken up in 1 ml of PBL for analysis on the flow cytometer.

Staining of PHA-stimulated PBL for surface antigens

PHA-stimulated PBLs are sedimented at 500 x G for 5 min at room temperature. The supernatant culture medium is poured off and the pellets allowed to drain. The cells are then washed with 5 ml of PBL (0.15 M NaCl, 0.02 M sodium phosphate, pH 7.2), and then sedimented at 500 x g or 5 min at room temperature. The supernatant is poured off and 100 ml of PBL is added, the cells resuspended and incubated with 10 ml each of CD3-phcoerythrin, (Dako, Burlingame, CA) and CD8-phcoerythrin/cyanin 5, (Caltag Laboratories, South San Francisco, CA) for 15 min in reduced light at room temperature. Five ml of PBL is added and the cells resedimented as described above. The cell pellet is drained and resuspended in 1 ml of PBL for flow cytometric analysis.

Flow cytometric analysis

Data acquisition is performed on a FACSort flow cytometer (Becton/Dickinson, San Jose, CA) equipped with a

15mW argon ion laser and a doublet discrimination module using the LYDYD II software (Becton/Dickinson). The instrument is calibrated prior to data acquisition using unstained PBLs. Fluorescence compensation is performed electronically using CD4-FITC/CD8-PE and CD8-PE/Cy5 stained peripheral blood lymphocytes. For each measurement, at least 20,000 events are collected and information on them stored. For data analysis, the PAINT-A-GATE PRO and the ATTRACTORS software (Becton/Dickinson) are used after converting Consort32 data files into the Apple 7.1 system through the CONSORT FILE EXCHANGE software (Becton/Dickinson).

Assay of TNF-a

Recent evidence suggests that thalidomide may suppress the production of TNFa by monocytes and that IL-2 production is increased in normal monocytes from individuals treated with thalidomide. For assaying the effects of the thalidomide derivatives on TPA-induced TNF-a, the Amersham kit is used for the ELISA testing of human TNFa in HL60 cells. Various concentrations of the test thalidomide derivatives (0.1 - 100 nM) are added to the cell cultures. TNF-a is determined by ELISA assay as recommended by the supplier.

Determination of IL-2 production

IL-2 production in human peripheral blood lymphocytes (HPBL) is be monitored by ELISA assay using a monoclonal anti-IL-2 antibody from DKO. 2 to 4 x 10^6 HPBLs stimulated with PHA for 48 hours are incubated with the monoclonal antibody solution (10 ml) for 20 min in an ELISA assay as described by the supplier.

What is claimed is:

1. A method for treating a patient comprising:

providing a composition comprising a thalidomide prodrug; and

administering the thalidomide prodrug to the patient.

2. The method according to claim 1, wherein the prodrug comprises a thalidomide component having bound thereto a dipeptide comprising the sequence X-pro; wherein "X" represents an amino acid and "pro" represents the imino acid proline.

3. The method according to claim 1, wherein the prodrug comprises a thalidomide component having bound thereto a dipeptide comprising the sequence ala-pro.

4. The method according to claim 1, wherein the prodrug comprises a thalidomide molecule having bound thereto a dipeptide comprising the sequence lys-pro.

5. The method according to claim 1, wherein the prodrug comprises a thalidomide molecule having bound thereto a dipeptide comprising the sequence gly-pro.

6. The method according to claim 1, wherein the composition comprises a thalidomide prodrug and a pharmaceutically acceptable carrier.

7. A composition useful for selectively treating aphthous ulcers, wasting, tuberculosis, dementia, fevers, fatigue, HIV infection and the loss of functional CD4 T cells, comprising a thalidomide component having bound thereto a dipeptide selected from the group consisting of ala-pro, gly-pro and lys-pro.

8. The composition according to claim 7, wherein said dipeptide comprises the sequence X-pro; and wherein "X" represents an amino acid.

9. The composition according to claim 7, wherein said dipeptide comprises the sequence ala-pro.

10. A method for making a thalidomide prodrug, comprising providing thalidomide and binding thereto proline and a second amino acid such that the second amino acid is the N-terminal amino acid and proline is in the penultimate position to the N-terminal amino acid.

11. The method according to claim 10, wherein the second amino acid is alanine.

12. The method according to claim 10, wherein the second amino acid is glycine.

13. The method according to claim 10, wherein the second amino acid is lysine.

INTERNATIONAL SEARCH REPORT

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PCT/US97/09421

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :A61K 31/435
US CL :514/323
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/323

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

none

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG, MEDLINE, EMBASE, AIDSLINE, DERWENT WORLD PATENT, thalidomide, prodrug, analog, proline,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,443,824 A (PIACQUADIO) 22 August 1995, see entire document.	1-13
Y	US 4,709,014 A (TAMAOKI) 24 November 1987, see entire document.	1-13
Y	US 5,434,170 A (ANDRULIS, JR.) 18 July 1995, see entire document.	1-13
Y	MCHUGH et al. The Immunosuppressive Drug Thalidomide Induces T Helper Cell Type 2 (TH2) And Concomitantly Inhibits TH1 Cytokine Production In Mitogen- And Antigen-Stimulated Peripheral Blood Mononuclear Cultures. Clinical and Experimental Immunology. 1995, Vol. 99, pages 160-167, see entire document.	1-13

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
04 AUGUST 1997	21 AUG 1997

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